



Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin

Marion Spée^{a,*}, Michaël Beaulieu^a, Antoine Dervaux^a, Olivier Chastel^b, Yvon Le Maho^a, Thierry Raclot^a

^a Institut Pluridisciplinaire Hubert Curien (IPHC), Département d'Ecologie, Physiologie, Ethologie (DEPE), UMR 7178 CNRS-UdS, 23 rue Becquerel, 67087, Strasbourg Cedex 2, France

^b Centres d'Etude Biologiques de Chizé (CEBC), CNRS, F-79360, Villiers en Bois, France

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ABSTRACT

According to life-history theory, long-lived birds should favor their survival over the current reproductive attempt, when breeding becomes too costly. In seabirds, incubation is often associated with spontaneous long-term fasting. Below a threshold in body reserves, hormonal and metabolic shift characteristics of a switch from lipid to protein utilization (phase III, PIII) occur. These metabolic changes are paralleled by nest abandonment and stimulation of refeeding behavior. Parental behavior is then under control of two hormones with opposite effects: corticosterone (CORT) and prolactin which stimulate foraging and incubation behavior, respectively.

The aim of this study was to determine the respective role of these two hormones in nest abandonment by Adélie penguins. To this end, plasma hormone levels were measured before egg-laying and at departure from the colony (i.e. when birds were relieved by their partner or abandoned their nest), and related to nutritional state and incubation success.

We found that males abandoning their nest in PIII presented high CORT levels and low prolactin levels. Interestingly, males which presented high plasma levels of prolactin in PIII did not abandon. We show that although CORT is the first hormone to be affected by prolonged energy constraints, the combined effects of high CORT and low prolactin levels are necessary for parents to favor self-maintenance and abandon the nest. We provide insights into time-course changes of the endocrine profile as PIII proceeds and report that reaching proteolytic late fasting is not sufficient to induce nest abandonment in a long-lived bird.

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Introduction

Life-history theory predicts that during the breeding season long-lived birds should favor their own survival over the current reproductive attempt when energetic constraints become too serious (Stearns, 1992). In several bird species, breeding is associated with fasting because foraging might not be possible during incubation. For instance, long fasting periods are common in seabirds, since they feed exclusively at sea whereas breeding occurs on land. To cope with such reproductive patterns (i.e. breeding associated with sustained fasting bouts), biparental species may adopt one of two strategies: either (1) one parent may remain constantly on the nest being provisioned by its partner (e.g. raptors, Donozar et al., 1992) or (2) both parents may alternate between nest attendance and foraging at sea (e.g. seabirds, Tveraa et al., 1997). The parent assuming incubation duty must have previously accumulated sufficient body reserves to sustain long-term fasting. However, birds are likely to discontinue incubation when energy reserves are reaching a critical point of exhaustion. Indeed, below a

threshold in body reserves, birds enter phase III of fasting (PIII). At this stage, a metabolic shift occurs: uric acid levels increase (indicating protein catabolism), while plasma levels of β -hydroxybutyrate (BOHB) decrease, indicating a reduction in the utilization of lipids as the main energy substrate (Cherel et al., 1988; Robin et al., 1998). Moreover, behavioral changes, such as an increase in locomotor activity (Robin et al., 1998) and nest abandonment (Groscolas et al., 2008) have been reported from birds in PIII, indicating that when body reserves are close to exhaustion, the promotion of behaviors related to self-maintenance is favored.

Among the potential factors that may play a role in redirecting breeding behavior (e.g. incubation) to behavior ensuring survival (e.g. foraging activity), hormones can offer great insights into the mechanisms that mediate some life-history trade-offs (Sinervo and Svensson, 1998). Corticosterone (CORT), the major avian glucocorticoid, has been associated with the promotion of an emergency life-history stage, inducing behavior that ensures survival (Wingfield et al., 1998). This suggestion is supported by the finding that CORT levels increase in PIII (Cherel et al., 1988; Robin et al., 1998), and stimulates protein catabolism (Challet et al., 1995). Moreover, experimental administration of CORT mimics the fasting-induced rise in locomotor activity in laboratory rats (Challet et al., 1995) and in Adélie penguins (Spée et al.,

* Corresponding author. Fax: +33 3 88 10 69 06.

E-mail address: marion.spee@c-strasbourg.fr (M. Spée).

unpublished data). In addition, CORT levels have been shown to be markedly increased in PIII king penguins *Aptenodytes patagonicus* abandoning their egg, suggesting that this hormone plays an important role in the decision to give up a breeding attempt by stimulating the refeeding drive (Groscolas et al., 2008).

The pituitary hormone prolactin, on the other hand, has the opposite effect to CORT with respect to parental behavior and is known to stimulate incubation and brooding behavior in birds (Buntin, 1996; Youngren et al., 1991). Consequently, it is important to investigate the levels of both hormones (CORT and prolactin), when studying the physiological mechanisms that underlie parental behavior in birds. In penguins, prolactin secretion seems to be endogenously programmed on a long-term basis, being poorly influenced by external stimuli (Garcia et al., 1996; Lormée et al., 1999; Vleck et al., 2000a). Prolactin levels increase during courtship, peak in mid-incubation, and remain elevated until the end of chick brooding (Vleck et al., 1999). However, a decrease in prolactin levels has been reported in king penguins that abandoned their nest during PIII (Cherel et al., 1994; Groscolas et al., 2008), suggesting that prolactin secretion could be modulated by energy constraints on a short term basis. Such a drop in prolactin levels might decrease the incubation drive and favor nest abandonment.

Studies to date indicate that the induction of a refeeding signal leading to nest abandonment is related to PIII of fasting (Groscolas et al., 2008). At this stage, the increase in plasma CORT levels and the decrease in prolactin levels seem to play a role in motivating the parent's decision to abandon the nest, by stimulating the drive to refeed and by diminishing the drive to incubate, respectively (Groscolas et al., 2008). However, the respective role of these two hormones in stimulating nest desertion remains unclear. Moreover, whether prolonged energy constraints affect CORT and prolactin levels concomitantly or successively remains to be determined. In other words, whether reaching proteolytic PIII during late fasting is sufficient to induce nest abandonment alone in long-lived birds requires further consideration.

In penguins, both males and females take part in incubation and brooding duties, alternating fasting on land and foraging at sea. After egg-laying, females return to sea in order to forage and replenish their energy reserves while males take on the first incubation shift. In the present study, we investigated the

nutritional and hormonal patterns of Adélie penguins (1) at the beginning of the incubation fast and (2) when males departed from the colony to forage at sea. Male penguins left the colony either because they were relieved by their partner or because they abandoned the nest. The nutritional state of birds was determined from their body mass and from plasma metabolites, which are known to be good indicators of the nutritional state in free-living animals (Jenni-Eiermann and Jenni, 1998). Recording these nutritional parameters in parallel with plasma CORT and prolactin levels, we determined whether the entrance into PIII was consistently associated with nest abandonment and refeeding. We also assessed the respective role of CORT and prolactin in the induction of this behavioral shift. Moreover, we examined whether the entrance into PIII was the consequence of a lower body mass at the beginning of the fast or if these males had fasted for a longer period. Indeed, the body condition of male penguins at the beginning of the fast seems to be an important predictor of incubation success (Vleck and Vleck, 2002).

Methods

The study was conducted in Dumont d'Urville Station (66°40'S, 140°01'E), Adélie land, Antarctica, during the 2005–2006 austral summer and was supported by the Ethics Committee of the French Polar Institute Paul Emile Victor (IPEV).

Sampling state

During the study period, we followed 92 pairs of Adélie penguins. Birds were captured on two occasions (see Fig. 1).

First, both members of a pair were captured between pair formation and egg-laying (pre-laying stage). Blood was collected from the wing vein within 5 min of initial capture since it has been shown that handling durations <5 min have no effect on baseline CORT levels in Adélie penguins (Vleck et al., 2000b). Samples were subsequently transferred into pre-treated tubes (using heparin or EDTA) and centrifuged at 4 °C (5000 rpm for 10 min). The plasma was then collected and kept frozen in aliquots at –20 °C until subsequent analyses. All birds were weighed to the nearest 2 g using an Ohaus electronic precision balance and individually marked with a number

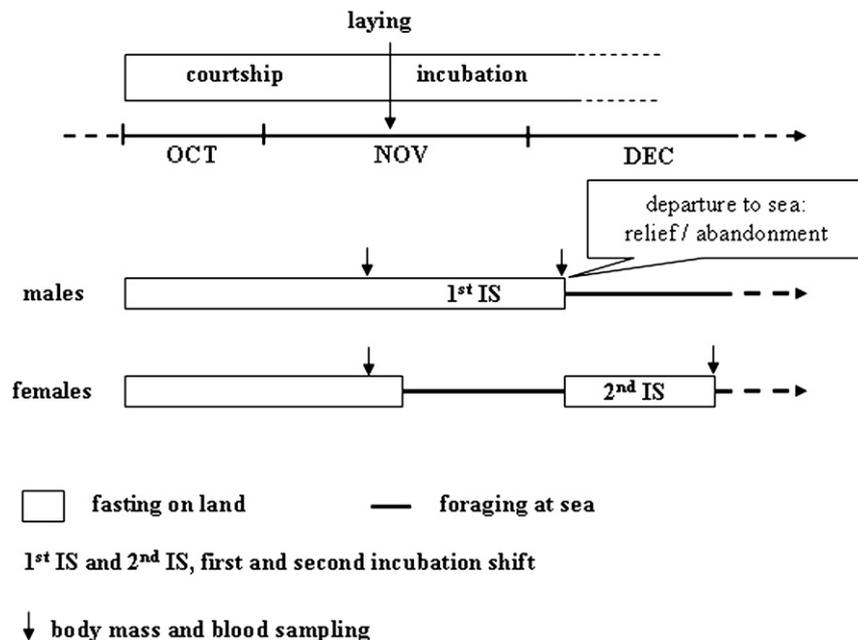


Fig. 1. Study protocol. Birds were weighed and sampled for blood on two occasions: at the pre-laying stage (males and females) and when departing to sea for refeeding (at the end of the first and second incubation shift for males and females, respectively). See *Methods* section for details.

painted on their chest using Nyanzol D. Sex was determined by observing nest attendance patterns (males usually take on the first incubation shift; Ainley et al., 1983) and by measuring plasma lipemia (females exhibit higher plasma lipemia before egg-laying than males, Beaulieu et al., 2010; Kern et al., 2005). The assigning of sex by these methods has already been used by Vleck and colleagues (2000a).

Nests were observed in 3–4 h intervals to determine bird attendance, egg-laying dates, number of eggs in the nest, and to note relief by the partner or nest abandonment. This study focused on the overall relatively small proportion of male penguins which were likely to reach PIII of fasting and were therefore prone to abandon their nest towards the end of the first incubation shift. Knowing the body mass of these birds at the pre-laying stage, the rate of daily body mass loss (0.052 kg/day, Chappell et al., 1993; 0.042 kg/day, Vleck and Vleck, 2002), and their critical body mass (3.5 kg, Cockrem et al., 2006), it was possible to estimate the approximate date at which birds should reach PIII, and were thus likely to desert their nest. Nest observations were especially important for the males at this point in time and were carried out on an hourly basis.

Second, males were recaptured at the end of the first incubation shift, when they left the colony to forage at sea (when their partner had returned or when they abandoned the nest). Birds were weighed and blood samples were taken. We also recaptured females when they left the colony to refeed at sea at the end of the second incubation shift.

We captured and weighed most males when they left the colony but did not systematically sample blood in all of these males 1) to avoid excessive manipulation and subsequent disturbance; 2) because the procedure was time-consuming; and 3) because many birds with a similar body mass and, thus, a similar nutritional state (PII of fasting) had already been sampled.

In total, during the first capture, 92 pairs were marked, weighed and measured (bill and flipper) but only 48 males and 48 females were sampled for blood. We were able to recapture and weigh most of the marked males when they left the colony at the end of the first incubation shift. At this stage, only 44 males were sampled for blood, of which 27 had already been sampled at the first capture. Of these 27 birds, four males abandoned their nest. In addition, 53 females were recaptured and weighed at the end of the second incubation shift and 27 of them were sampled for blood.

Plasma analysis

Metabolites

Concentrations of uric acid and β -hydroxybutyrate (β OHB) were measured by the enzymatic colorimetric method using commercial kits (uric acid: Sigma Diagnostics; β OHB: Randox). The measurement was conducted on undiluted plasma (uric acid: 25 μ l; β OHB: 20 μ l).

Hormones

CORT concentrations were determined by a quantitative competitive sandwich enzyme immunoassay technique (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé (CEBC; France). Pooled plasma samples of Adélie penguins produced a dose response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance). For prolactin measurements, intra and inter-assay variations were 6% and 9%, respectively. For corticosterone determinations, these variations were 5% and 7%, respectively.

Determination of fasting phases and statistics

To determine whether birds were either in PII or PIII at the end of their first incubation shift, we used a Principal Component Analysis

(PCA) specifying body mass, uric acid levels (reflecting protein catabolism) and β OHB concentrations (reflecting lipid utilization) as variables in the analysis. These parameters are indeed known to be good indicators of the nutritional state of free-living birds (Jenni-Eiermann and Jenni, 1998). PCA was conducted with R (2.8.1) using the FactorMineR package. The number of dimension selected for analysis was reduced to one axis following the Kaiser criteria, i.e. only considering axes with an Eigenvalue > 1 .

Birds were divided into three groups: ePII (males at the end of PII which were relieved by their partner, $n = 18$), PIII + re (males in PIII relieved by their partner, $n = 5$) and PIII + ab (males in PIII which abandoned their nest before having been relieved by their partner, $n = 4$). General linear mixed models (GLMMs) were used to compare plasma parameters between groups. We included individuals as a random factor and "sampling period" (at the pre-laying stage and at departure to sea), "group" (ePII, PIII + re and PIII + ab) and their interaction as fixed factors, the "sampling period" being the repeated measure. Normality was assessed using a Shapiro–Wilk test. When normality was not met, a generalized estimated equation (GEE) was used. To compare body mass at the pre-laying stage and daily body mass loss, we used a general linear model (GLM). The clutch size and the duration of fasting were compared using a generalized linear mixed model (GzLM) with a Poisson distribution. Post-hoc comparisons were made using Bonferroni tests. We used Student's *t*-test to compare PC1-scores between birds in PIII + re and birds in PIII + ab.

Analyses were conducted using Minitab 15 software and SPSS 16.02 (SPSS Inc., Chicago, Ill., USA). Results are expressed as means \pm S.E. and significance level was set at $\alpha = 0.05$.

Results

Males during the first incubation shift

Mean body mass of the 27 males captured at the pre-laying stage was 5.05 ± 0.11 kg (range: 4.13 to 6.61 kg). During incubation, 23 of these males were relieved by females and only four abandoned their nest.

The first dimension of the PCA (with body mass, uric acid and β OHB as variables) explained 68.9% of the total variation. However, when we corrected body mass for morphological size, the PCA only explained 67.1% of the total variation. Consequently, we only consider here the PCA which included body mass alone and did not correct for morphological size. According to the individuals' factor map (and taking into account only the first dimension), we were able to distinguish two groups having distinct fasting status among birds that left the colony to refeed at sea: penguins in PII and penguins in PIII (PIII + re and PIII + ab). The frequency distribution of the PC1-scores of these males is shown in Fig. 2. We found no significant difference between PC1 scores of birds in PIII + re and those of birds in PIII + ab (PIII + re = -1.36 ± 0.16 and PIII + ab = -2.13 ± 0.70 ; $t = 1.07$, $df = 3$, $p = 0.36$).

Finally, of the 23 males relieved by females, 18 were in ePII, while five were in PIII (PIII + re). However, all the birds that abandoned their nest were in PIII (PIII + ab).

Body mass at the pre-laying stage, clutch size, body mass loss and fasting duration

Male body mass at the pre-laying stage (i.e. at first capture) differed significantly between groups ($F_{2, 22} = 11.71$, $p < 0.001$; Table 1). Penguins that abandoned their nest ($p = 0.001$) and birds that were relieved in PIII ($p = 0.009$) had significantly lower body mass during the pre-laying period than birds that left the colony in ePII (16% and 12% lower, respectively). Hence, penguins in PIII had similar body masses at this stage, regardless of the outcome of the first incubation shift ($p > 0.99$).

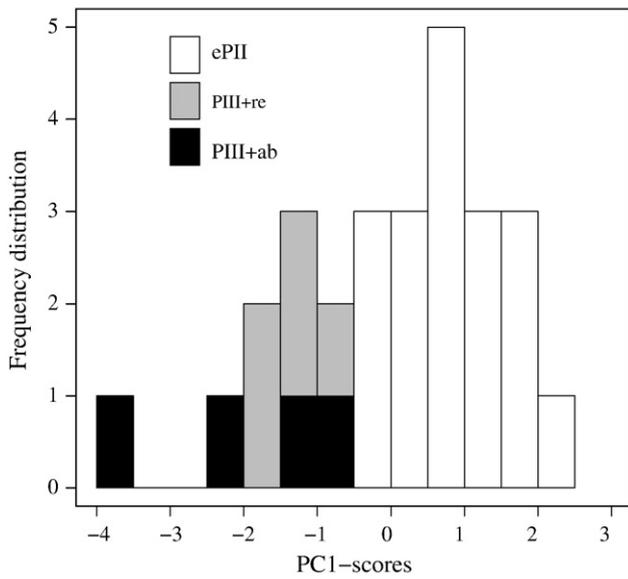


Fig. 2. Frequency distribution of PC1-scores of male Adélie penguins that fall into ePII (males at the end of phase II relieved by their partner, white bars, $n = 18$), PIII + re (males in phase III relieved by their partner, grey bars, $n = 5$) or PIII + ab (males in phase III that abandoned their nest, black bars, $n = 4$) at the end of the first incubation shift.

Penguins had similar clutch size, regardless of whether they fell into ePII (1.88 ± 0.08), PIII + re (1.80 ± 0.20) or PIII + ab (2.00 ± 0.00) at the end of the first incubation shift (Wald $\chi^2 = 0.05$, $df = 2$, $p = 0.98$).

Fasting duration for males between the first and the second capture was similar for all groups (Wald $\chi^2 = 1.64$, $df = 2$, $p = 0.44$; Table 1). Daily body mass loss of males was not significantly different between groups ($F_{2, 22} = 1.37$, $p = 0.28$; Table 1).

Plasma levels of metabolites

Uric acid levels of birds in PIII (PIII + re and PIII + ab) were affected by the sampling period ($F_{1, 3} = 13.4$, $p = 0.04$; Fig. 3A). Indeed, birds in PIII + re and PIII + ab exhibited higher uric acid levels when departing to sea than during the pre-laying stage. However, uric acid concentrations were not affected by the group (PIII + re and PIII + ab; $F_{1, 3} = 1.26$, $p = 0.35$) and by the interaction group* sampling period ($F_{1, 3} = 1.26$, $p = 0.35$).

In addition, β OHB levels of birds in PIII were influenced by the period of sampling ($F_{1, 6} = 11.2$, $p = 0.02$), with birds in PIII + re and PIII + ab showing lower β OHB levels when departing to sea than during the pre-laying stage. However, β OHB concentrations were not affected by the group (PIII + re and PIII + ab; $F_{1, 7} = 0.003$, $p = 0.96$) and by the interaction between group*period of sampling ($F_{1, 6} = 0.49$, $p = 0.51$).

Table 1
Profile of breeding male Adélie penguins according to reproductive performance and nutritional status at the end of the first incubation shift.

Nutritional state	Relief by female		Abandonment
	ePII	PIII + re	PIII + ab
Body mass at the pre-laying stage (kg)	5.28 ± 0.08^a	4.67 ± 0.16^b	4.44 ± 0.27^b
Fasting duration between first and second capture (days)	20.6 ± 0.9	21.0 ± 1.7	17.7 ± 2.8
Body mass loss (g/day)	55.8 ± 1.7	55.8 ± 3.2	62.0 ± 3.4

ePII, males at the end of phase II relieved by females, $n = 18$; PIII + re, males in phase III relieved by females, $n = 5$; PIII + ab, males in phase III that abandoned their nest, $n = 4$. Results are means \pm S.E. For the body mass at the pre-laying stage, values that do not share the same superscript letter are significantly different. For the other parameters, no significant differences were detected between groups.

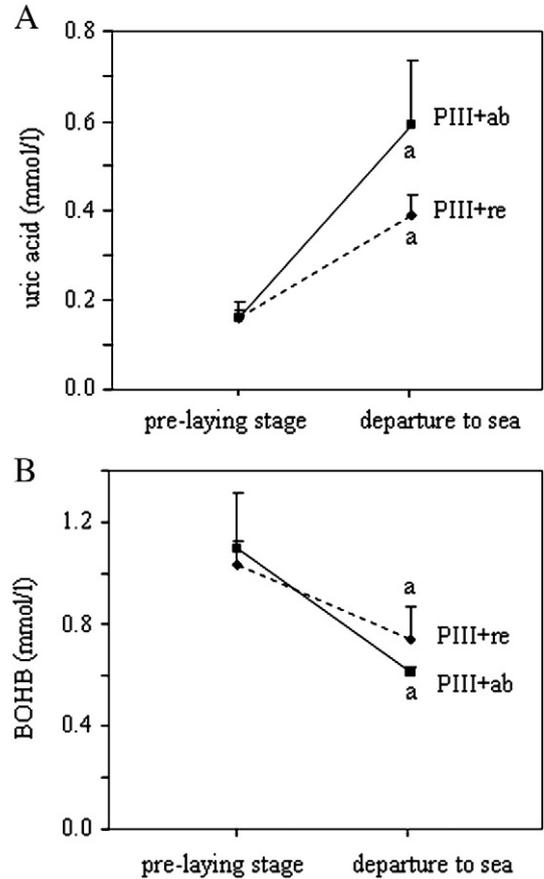


Fig. 3. Plasma levels of uric acid (A) and β OHB (B) of breeding male Adélie penguins in PIII at the pre-laying stage and when departing to sea (relief by their partner or nest abandonment). PIII + re: males in phase III relieved by females, $n = 5$; PIII + ab: males in phase III that abandoned their nest, $n = 4$. Results are means \pm S.E. Bars not sharing the same superscript letter are significantly different.

Plasma levels of hormones

CORT levels were affected by the sampling period (Wald $\chi^2 = 28.5$, $df = 1$, $p < 0.001$; Fig. 4A), the group (Wald $\chi^2 = 18.5$, $df = 2$, $p < 0.001$) and their interaction (Wald $\chi^2 = 13.4$, $df = 2$, $p = 0.001$; Fig. 4A). CORT levels at the pre-laying stage were similar for all penguins ($p > 0.99$ for each comparison). At departure to sea, birds in PIII + re and PIII + ab had significantly higher CORT levels than at the pre-laying stage ($p = 0.004$ and $p = 0.01$, respectively), while we found no difference for birds in ePII ($p = 0.19$). Moreover, plasma levels of CORT were not significantly different for birds in PIII + re and PIII + ab ($p > 0.99$).

Prolactin levels were influenced by the group ($F_{2, 24} = 6.59$, $p = 0.005$; Fig. 4B) and by the interaction between group* sampling period ($F_{1, 23} = 20.5$, $p < 0.001$) but were not affected by the sampling period ($F_{1, 24} = 0.27$, $p = 0.61$). Prolactin levels at the pre-laying stage were similar for all birds ($p > 0.99$ for each comparison). At departure to sea, prolactin levels of penguins in PIII + ab were 62% lower than at the pre-laying stage ($p < 0.001$). Interestingly, penguins in PIII + ab had 70% lower prolactin concentrations than birds in PIII + re ($p < 0.001$), while they were in a similar nutritional state (enhanced proteolysis).

Females at the pre-laying stage and at the end of the second incubation shift

At the pre-laying stage, mean body mass of females was 4.4 ± 0.1 kg (ranging from 3.67 to 6.11 kg). Their plasma levels of uric acid, CORT, and prolactin were 0.11 ± 0.01 mmol/l, 4.2 ± 0.7 ng/ml, and 91 ± 11 ng/ml, respectively.

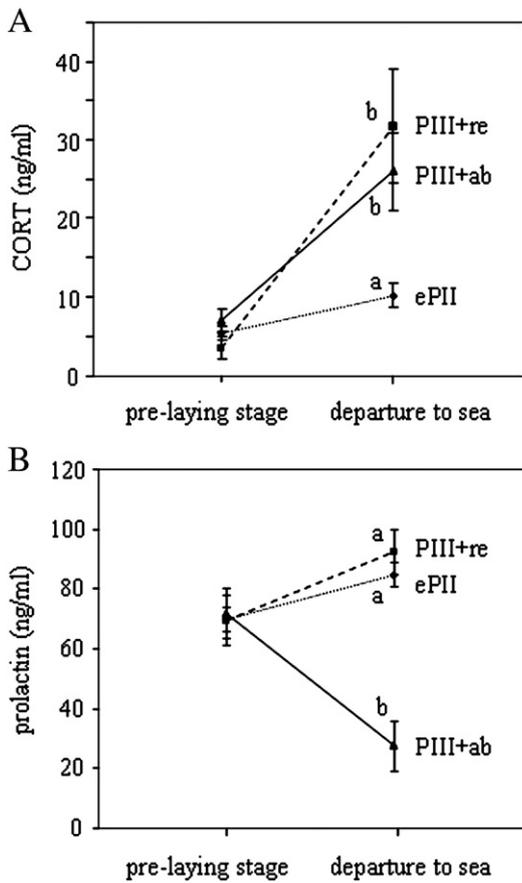


Fig. 4. Plasma levels of CORT (A) and prolactin (B) of breeding male Adélie penguins at the pre-laying stage and when departing to sea (relief by their partner or nest abandonment). ePII: males at the end of phase II relieved by their partner, $n = 18$; PIII + re: males in phase III relieved by their partner, $n = 5$; PIII + ab: males in phase III that abandoned their nest, $n = 4$. Results are means \pm S.E.

At the end of the second incubation shift, all females that departed to sea were relieved by their respective mate. Of the 27 females captured at this time, 25 were in ePII, while two were in PIII (PIII + re).

Given the small sample size of birds in PIII ($n = 2$), statistical comparison between females in ePII and females in PIII + re was not performed. However, the trend observed for each parameter measured is indicated in Table 2. We found that females in PIII + re tended to have lower body masses, higher plasma uric acid and CORT levels than females in ePII. By contrast, prolactin levels tended to be similar for all females.

Discussion

Our study shows that reaching proteolytic PIII during late fasting is not sufficient to induce nest abandonment in a long-lived seabird, the Adélie penguin. This finding indicates a decoupling between the metabolic status of a bird and its behavioral response. We found that whereas all breeding males presented elevated CORT levels in PIII, only those which also presented high prolactin levels did not abandon incubation. Conversely all males presenting low prolactin levels in PIII abandoned the nest and left to forage at sea. In addition, penguins which entered PIII did so because their body mass at the beginning of the incubation fast was low, suggesting that these birds might have been less experienced breeders or that they might have presented poor foraging abilities at the time.

Table 2
Profile of breeding female Adélie penguins according to nutritional status at the end of the second incubation shift.

Nutritional state	ePII	PIII + re	Trend (PIII + re vs. ePII)
Fasting duration (2nd shift) (days)	12.71 ± 0.38	14.50 ± 0.50 (14 and 12)	→
Body mass (kg)	4.04 ± 0.07	3.08 ± 0.05 (3.03 and 3.13)	\
Uric acid (mmol/l)	0.18 ± 0.02	0.33 ± 0.03 (0.31 and 0.36)	/
CORT (ng/ml)	10.03 ± 1.67	20.41 ± 3.65 (16.75 and 24.06)	/
Prolactin (ng/ml)	96.99 ± 2.01	95.15 ± 6.45 (88.71 and 101.6)	→

ePII, females at the end of phase II relieved by males, $n = 25$; PIII + re, females in phase III relieved by males, $n = 2$. CORT, corticosterone. Results are means \pm S.E.

Nutritional state and hormonal status at relief/abandonment

According to the result of the PCA, we found that all abandoning penguins were in PIII (PIII + ab). Birds showed increased uric acid levels and decreased β OHB concentrations (Fig. 3), indicating a metabolic shift from lipid towards protein utilization. Moreover, their body mass was lower than the 3.5 kg critical body mass threshold that typically signals the entrance of male Adélie penguins into PIII (Cockrem et al., 2006). Our results for Adélie penguins are similar to the situation found in king penguins, where nest abandonment occurs during the late stage of fasting (Groscolas et al., 2008). Given their long life span, the optimal strategy for an incubating penguin entering PIII may be to abandon the current reproductive effort in favor of its own survival and thereby ensure future reproductive attempts. Surprisingly however, we found that some birds in a similar nutritional state (i.e. in PIII) did not abandon their nest but were relieved by their partner (PIII + re). This result suggests a decoupling between the metabolic status and the behavioral response.

As expected, abandoning birds in the current study had high levels of CORT and low concentrations of prolactin (Fig. 4), indicating a stimulation of the refeeding drive, while the incubation drive was depressed. Such hormonal patterns seem to be the characteristic for the process of nest abandonment in penguins, as deserting king penguins show comparable changes in CORT and prolactin levels (Groscolas et al., 2008). We found that prolactin levels were sharply decreased in abandoning birds, while they remained high in birds in PIII that did not desert their nest. Hence, in addition to increased levels of CORT, decreased prolactin levels are also required to induce nest abandonment in Adélie penguins. We also found some females in PIII which did not desert their nest at the end of the second incubation shift (Table 2). These females presented high CORT and prolactin levels, indicating that such a situation is not restricted to males at the end of the first incubation shift.

Our findings suggest that the hormonal changes occurring in PIII would be dynamic. The increase in the refeeding drive orchestrated by increased CORT levels seems to precede the decrease in the incubating drive, which is modulated through a decline in prolactin concentrations.

Why do only some penguins in PIII abandon their nest?

Three main reasons can be proposed to explain why prolactin levels only declined in abandoning penguins.

First, assuming that baseline prolactin concentrations are indicative of the amount and quality of parental care provided (Angelier et al., 2007a,b; Angelier and Chastel, 2009), we can hypothesize that the parental care provided by abandoning penguins was already reduced at the start of incubation, when compared to that of non-

abandoning birds. Hence, prolactin levels would already be low at the beginning of the incubation fast in abandoning birds. However, we show that the three groups of penguins had similar prolactin levels at the pre-laying stage (Fig. 4B), supporting the view that all birds were similarly motivated to incubate.

Second, the differences observed in prolactin patterns between abandoning birds and birds in PIII which were relieved by their mates may be explained by a differential regulation of prolactin secretion in response to the stress caused by prolonged fasting. Factors that modulate the prolactin response to stress are usually examined using a standardized stress protocol (Angelier et al., 2007a, 2009; Chastel et al., 2005). Although it could be argued that fasting during incubation is predictable and should therefore not be associated with stress, the prolonged energy constraint that induces birds to enter PIII most likely exposes birds to physiological stress. This view is supported by increased CORT levels at this stage of fasting (Fig. 4A). Moreover, because parental effort has been shown to modulate the prolactin response to stress (chick-rearing vs. failed black-legged kittiwakes *Rissa tridactyla*; Chastel et al., 2005; Angelier and Chastel, 2009 for a review), we can hypothesize that penguins should be more reluctant to abandon two eggs than one. However, clutch size of penguins that left the colony to forage at sea in ePIII, PIII + re, and PIII + ab was similar.

On the other hand, there is evidence that the prolactin response to stress is modulated by age (Angelier et al., 2007a). In fact, young breeding Adélie penguins are reportedly more likely to desert their nest than older ones (Davis and McCaffrey, 1986). In this respect, we can hypothesize that penguins in PIII + re were older breeders (which were more resistant to stressful situations) and that their prolactin levels remained elevated when CORT increased. Future studies performed on birds of known age or that provide information about the estimated relative age (Hausmann et al., 2003a,b) of abandoning penguins will allow testing this hypothesis.

Third, we can hypothesize that birds in PIII + ab were in more advanced in PIII than penguins in PIII + re, i.e. they could have been in PIII for a longer period. Results from abandoning king penguins are in line with this hypothesis. Groscolas and colleagues found that body mass in abandoning king penguins was about 1 kg below the critical body mass value, with the total duration for the egg abandonment process ranging from 20 h to 5 days (Groscolas et al., 2000). Thus, entrance into an emergency life-history stage driven by elevated CORT levels that redirect behavior towards survival (Wingfield et al., 1998) can take a long time to develop. We found no significant differences in body mass loss and PC1-scores between birds in PIII that abandoned the nest and birds in PIII that were relieved by females, despite a tendency for abandoning birds. Due to our low sample size ($n = 5$ for birds in PIII + re and $n = 4$ for birds in PIII + ab), we cannot provide any statement based on this non-statistical significance. Thus, we are only able to suggest that abandoning birds could have been more advanced in PIII than birds which were relieved.

Do prolactin levels have to reach a threshold concentration in abandoning penguins?

In the context of nest abandonment as an adaptive behavior promoting survival, CORT could affect endocrine mechanisms involved in parental activities, such as prolactin. Indeed, CORT and prolactin are mechanistically linked. For birds it has been shown that an experimentally induced rise in CORT levels leads to a decrease in prolactin levels (Angelier et al., 2009; Criscuolo et al., 2005) and it has been suggested that the effects of CORT on the expression of parental behavior may be mediated through modulation of prolactin levels (Angelier et al., 2009). However, the inhibitory action of CORT on plasma prolactin seems to be complex, since prolactin levels have been shown to decrease only slowly and progressively in response to CORT manipulation (Angelier et al., 2009). For instance, an experimental increase in CORT levels in black-legged kittiwakes was

accompanied by a reduction in plasma prolactin concentrations and subsequently (from day 3 after treatment) by a reduction in nest attendance (Angelier et al., 2009), suggesting that prolactin levels probably need to reach a low threshold value to affect parental behavior. Interestingly, observed prolactin levels in abandoning penguins seem to be fairly similar (king penguins, Cherel et al., 1994; Groscolas et al., 2008; Adélie penguins, this study), reinforcing the idea that prolactin has to reach a threshold concentration, below which the drive to incubate is inhibited. Accordingly, we propose that abandoning penguins (PIII + ab) would have been in PIII for a longer period than birds which were relieved (PIII + re). Such a difference in timing is most likely required for CORT to decrease prolactin to a sufficiently low level. However, we cannot reject the hypothesis that birds in PIII + ab could be younger breeders that are less resistant to stressful situations and that their prolactin levels decreased as CORT increased.

The decline in prolactin levels could also be a consequence of the progressive decrease of attentiveness, driven by CORT, which precedes definitive nest desertion. Transitory abandonments, when birds leave the nest for progressively increasing durations and wander further and further away from their egg, have been reported in king penguins (Groscolas et al., 2000) and Red-footed booby *Sula sula* (Chastel and Lormée, 2002). Whether transitory abandonments occur in Adélie penguins, and whether the decline in prolactin levels precedes the decrease in attentiveness or merely follows, remains however unknown. A study conducted in meerkats *Suricata suricatta* supports the former course of events as high prolactin levels preceded the decision to engage in parental care (Carlson et al., 2006).

Conclusion – perspectives

In conclusion, we show that the PIII of fasting is not necessarily associated with nest abandonment in a long-lived seabird, the Adélie penguin. In some cases, we found a decoupling between the metabolic status (PIII) and the behavioral response of abandonment. Whereas CORT and prolactin are both involved in the induction of the refeeding signal that ultimately leads to nest abandonment, we propose that hormonal changes occurring in PIII would be dynamic, so that CORT and prolactin would be successively affected by prolonged energy constraints. In that respect, we hypothesized that CORT would act first and is not sufficient by itself to induce nest abandonment. To test this hypothesis, it would be interesting to examine the extent to which exogenous CORT mimics a PII–PIII transition and provokes nest abandonment, potentially mediated through a decline in prolactin concentrations. Similarly, to gain further insight into the role of prolactin in the control of incubation behavior, it would be of great interest to examine the effects of an experimental decrease in prolactin levels that is not accompanied by an increase in CORT levels. A better understanding of the interrelationships between marine resources, food availability, body condition, and breeding success in long-lived seabirds will require future studies to consider the proportion of birds reaching late fasting, the extent to which they desert their nest and the effect of environmental conditions on such parameters, in years to come.

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